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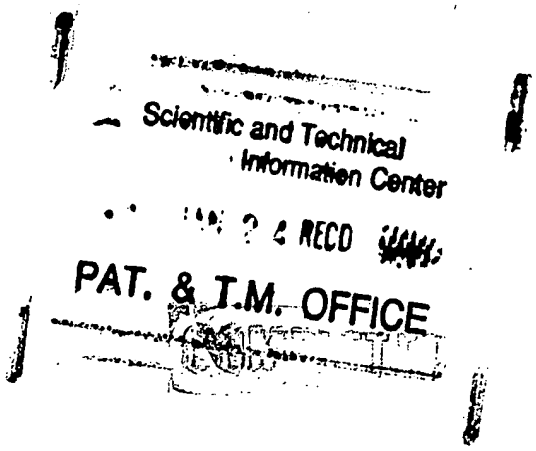

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The value of cerebrospinal fluid glycoprotein levels in the diagnosis of primary brain tumor

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OVER THE PAST few years, a number of investigators have been interested in the effect of disease on the chemistry of cerebrospinal fluid with particular regard to various protein-bound carbohydrates.

In a recent review of the chemistry of glycoproteins, Dische[1] indicated that glycoprotein can be divided into two general groups on the basis of the relative concentration of one or the other of the terminal carbohydrates of this molecule, that is, sialic acid and fucose. Roboz and associates[2] demonstrated the presence of fucose and sialic acid in the cerebrospinal fluid (CSF); we, therefore, initiated a study of the ratio of these two terminal carbohydrates of glycoproteins to the core carbohydrate, hexose, in this fluid.

MATERIALS AND METHODS

CSF was obtained mainly by lumbar puncture; a few samples were obtained during pneumoencephalogram or ventriculogram. There were no bloody or xanthochromic fluids used in this study. Those fluids not immediately analyzed were frozen and stored.

One of the difficulties in determining the concentration of any particular carbohydrate in the CSF is the large amount of glucose in this fluid. Since glycoproteins are large, nondialyzable molecules, it was found that all of the free sugar in the CSF could be removed by exhaustive dialysis overnight in the cold, leaving only the protein-bound carbohydrate in the dialysis bag. CSF sample of 3 to 5 ml. was dialyzed in this manner.

One-milliliter aliquots of the sample remaining in the dialysis bag were analyzed for three glycoprotein carbohydrates, namely, hexose, sialic acid, and fucose. Hexose was determined by the method of Roboz and associates[2] using borosulfuric acid and tryptophan. Sialic acid was determined by the method of Warren[3] using the periodate oxidation product extracted into cyclohexanone. Fucose was quantitatively analyzed by the method of Dische and Shettles[4] using the cysteine–sulfuric acid procedure.

The chromogens formed in these various determinations were found to be spectrally equivalent to the appropriate purified standards.

Fucose was also identified by paper chromatography and by the ability to quench the fucose-produced chromagen with water.[5]

Electrophoresis was performed on pooled CSF of nontumor patients obtained from the central hospital laboratory and on one specimen from a patient with glioblastoma multiforme. The fluids were first dialyzed exhaustively and lyophilized. Sixty milligrams of the lyophilized material from each of the respective samples were dissolved in 1 ml. of physiological normal saline. The Beckman electrophoretic apparatus then was used to separate the protein fractions electrophoretically on cellulose acetate membrane. The protein fractions were stained in a mixture of Ponceau S, trichloracetic acid, and sulfosalicylic acid. The carbohydrate portion of the glycoprotein was stained in periodic acid–Schiff. The resulting electrophoretic patterns were demarcated and

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TABLE 1

VALUES OF HEXOSE AND THE RATIO BETWEEN THE EXAMINED TERMINAL CARBOHYDRATE AS WELL AS THOSE TERMINAL TO THE CORE CARBOHYDRATE IN NORMAL AND ABNORMAL CONDITIONS

	Hexose (mg. %)	*Fucose/hexose (mg. %)*	*Sialate/hexose (mg. %)*	*Sialate/fucose (mg. %)*
Normal (13)				
Mean	1.12	0.54	0.50	0.91
Range	(1.00 - 1.20)	(0.33 - 0.70)	(0.45 - 0.59)	(0.65 - 1.39)
Convulsive disorders (23)				
Mean	*2.14	0.48	*0.22	*0.53
Range	(0.60 - 4.80)	(0.18 - 1.20)	(0.05 - 0.50)	(0.09 - 1.80)
Percent abnormal	83	22	83	61
Percent elevated	78	5	0	9
Multiple sclerosis (12)				
Mean	*2.86	0.51	*0.22	*0.35
Range	(0.90 - 8.00)	(0.33 - 0.82)	(0.03 - 0.45)	(0.10 - 0.78)
Percent abnormal	83	8	83	75
Percent elevated	83	8	0	0
Cerebral cortical atrophy (8)				
Mean	*1.27	0.79	*0.35	*0.455
Range	(0.34 - 7.80)	(0.47 - 1.3)	(0.04 - 0.63)	(0.07 - 0.81)
Percent abnormal	63	25	62	62
Percent elevated	63	25	25	0
Headaches (8)				
Mean	*2.42	0.58	*0.23	*0.43
Range	(0.90 - 4.80)	(0.19 - 0.80)	(0.02 - 0.57)	(0.04 - 0.78)
Percent abnormal	75	25	50	38
Percent elevated	75	0	0	0
Cerebrovascular disease (10)				
Mean	*2.03	0.54	*0.23	*0.46
Range	(1.20 - 3.60)	(0.28 - 0.91)	(0.07 - 0.33)	(0.24 - 0.90)
Percent abnormal	90	10	100	50
Percent elevated	90	10	0	0
Huntington's chorea (3)				
Mean	1.15	0.62	*0.29	*0.47
Range	(1.00 - 1.30)	(0.54 - 0.70)	(0.26 - 0.31)	(0.44 - 0.49)
Percent abnormal	0	0	100	0
Percent elevated	0	0	0	0
Subdural hematoma (3)				
Mean	*2.30	0.38	*0.34	*0.60
Range	(1.30 - 3.00)	(0.22 - 0.47)	(0.11 - 0.65)	(0.20 - 1.40)
Percent abnormal	67	0	33	33
Percent elevated	67	0	33	0
Chronic brain syndrome (3)				
Mean	*1.90	0.67	*0.24	*0.39
Range	(1.40 - 2.30)	(0.50 - 0.87)	(0.13 - 0.38)	(0.15 - 0.60)
Percent abnormal	100	33	67	100
Percent elevated	100	33	0	0
Miscellaneous (17)				
Mean	*2.12	0.52	*0.33	*0.53
Range	(0.90 - 4.30)	(0.23 - 1.30)	(0.05 - 1.40)	(0.07 - 1.50)
Percent abnormal	80	6	80	65
Percent elevated	71	6	12	6

*Significantly different from normal ($p<.05$)

TERMINAL CARBOHYDRATE AS
AL AND ABNORMAL CONDITIONS

e	Sialate/hexose (mg. %)	Sialate/fucose (mg. %)
	0.50	0.91
)	(0.45 - 0.59)	(0.65 - 1.39)
	*0.22	*0.53
)	(0.05 - 0.50)	(0.09 - 1.80)
	83	61
	0	9
	*0.22	*0.35
)	(0.03 - 0.45)	(0.10 - 0.78)
	83	75
	0	0
	*0.35	*0.455
)	(0.04 - 0.63)	(0.07 - 0.81)
	62	62
	25	0
	*0.23	*0.43
)	(0.02 - 0.57)	(0.04 - 0.78)
	50	38
	0	0
	*0.23	*0.46
)	(0.07 - 0.33)	(0.24 - 0.90)
	100	50
	0	0
	*0.29	*0.47
)	(0.26 - 0.31)	(0.44 - 0.49)
	100	0
	0	0
	*0.34	*0.60
)	(0.11 - 0.65)	(0.20 - 1.40)
	33	33
	33	0
	*0.24	*0.39
)	(0.13 - 0.38)	(0.15 - 0.60)
	67	100
	0	0
	*0.33	*0.53
)	(0.05 - 1.40)	(0.07 - 1.50)
	80	65
	12	6

ntegrated by the Beckman analytrol in the usual manner.

The fluids studied represented three groups of patients: [1] 13 normal patients receiving spinal anesthesia for normal deliveries, [2] 87 patients with various neurological diseases exclusive of tumors, and [3] 19 patients with brain tumor proved either by contrast studies, surgical biopsy, autopsy, or a combination of all three. Tumor suspects without the above verification were excluded.

The intracranial tumors were divided into 2 groups: primary and secondary. The primary group consisted of 3 astrocytomas, 5 glioblastomas, 1 ependymoma, 1 meningioma, 2 oligodendrogliomas, and 2 patients who unquestionably had tumors in whom tissue diagnosis was not available. One of the patients with an oligodendroglioma also had a pituitary adenoma. The secondary tumors included pituitary adenoma, leukemic lymphoma, metastatic melanosarcoma, and 2 metastatic carcinomas.

RESULTS

In Tables 1 and 2, the values of hexose were expressed as milligrams percent, the ratios of fucose to sialic acid, or sialic acid and fucose to hexose. The percent abnormal and percent elevated were calculated on a statistical basis.

Table 1 is based on the results in patients with specific neurologic diseases excluding brain tumors. Three of the four descending columns have mean values which are significantly different from normal. All ratios involving sialic acid are decreased in value while all hexose values are increased. The one remaining descending column (F/H) shows normal mean values throughout. It is interesting that the mean hexose in multiple sclerosis is the highest of this study but that the F/H ratio is within normal range.

Table 2 summarizes the normal and disease control groups in comparison to the intracranial tumors. Assuming that an F/H ratio in excess of the statistical range (0.80) is significantly elevated above normal (p less than .05), all of the diagnosed primary brain tumor fluids demonstrated an increase in this ratio. Two of the five secondary tumors exhibited an

TABLE 2

NONDIALYZABLE CARBOHYDRATE CONCENTRATION AND RATIOS IN HUMAN CEREBROSPINAL FLUID IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH BRAIN TUMORS

	Hexose (mg. %)	Fucose/hexose (mg. %)	Sialate/hexose (mg. %)	Sialate/fucose (mg. %)
Normal (13)				
Mean	1.12†	0.54	0.50†	0.91†
Range	(1.00 - 1.20)	(0.33 - 0.70)	(0.45 - 0.59)	(0.65 - 1.39)
Disease control (87)				
Mean	*2.02	0.50	*0.27	*0.51
Percent abnormal	78	14	80	70
Percent elevated	75	6	6	3
Intracranial tumors				
A. Primary tumors (14)				
Mean	1.71	*1.24†	0.44	*0.28†
Range	(0.60 - 2.70)	(0.85 - 2.20)	(0.14 - 0.68)	(0.11 - 0.43)
Percent abnormal	86	100	86	86
Percent elevated	71	100	0	0
B. Secondary tumors (5)				
Mean	1.60	0.78	*0.39	*0.51
Range	(0.70 - 2.90)	(0.45 - 1.13)	(0.19 - 0.53)	(0.31 - 0.78)
Percent abnormal	40	40	60	80
Percent elevated	40	40	0	0

*Significantly different from normal ($p<.05$)
†Significantly different from disease control ($p<.05$)

increase in the F/H ratio (a leukemic lymphoma and a melanosarcoma). It was interesting to note that the patient with an oligodendroglioma and pituitary adenoma had an increased F/H ratio while a patient with only a pituitary adenoma had a normal F/H ratio suggesting that the increase was due to the activity of the oligodendroglioma.

In the disease control group, 6% of the patients demonstrated a statistically elevated F/H ratio but only 4 patients had a value higher than that of the lowest valued primary brain tumor patient (0.85). One of these 4 patients was a brain tumor suspect clinically but who fulfilled none of the criteria for tumor diagnosis listed above. Another one of the patients had CNS lues and the remaining 2 had cortical atrophy secondary to cerebral arteriosclerosis.

The results in the first 2 tables indicate that, although the concentration of 3 nondialyzable carbohydrates varied considerably, the mean ratio of fucose to hexose is statistically identical in both the normal and disease control groups (0.54 ± 0.15 and 0.50 ± 0.14) and significantly elevated (1.24 ± 0.15) in the primary tumor group. The mean ratio is 0.78 in the secondary tumor group.

TABLE 3

THE QUANTITATIVE ELECTROPHORETIC PATTERNS OF THE CSF IN POOLED CONTROL AND A TUMOR PATIENT, AFTER DIALYSIS AND LYOPHILIZATION AND STAINING FOR PROTEINS AND CARBOHYDRATES (PAS)

	Protein		Carbohydrate (PAS)	
Fractions	*Control (%)*	*Tumor (%)*	*Control (%)*	*Tumor (%)*
Prealbumin	0	3	0	12
Albumin	61.5	60	22	38
Globulin	38.5	37	78	49

TABLE 4

COMPARISON OF CSF PROTEIN CONCENTRATIONS AND FUCOSE TO HEXOSE RATIOS IN PATIENTS WITH SUPERNORMAL PROTEIN CONCENTRATIONS FROM DISEASE CONTROL GROUP AND IN SIX PATIENTS WITH PRIMARY BRAIN TUMOR

Disease control		Primary tumor	
Protein (mg. %)	*F/H*	*Protein (mg. %)*	*F/H*
* 86	0.25		
* 90	*0.92	32	*2.2
* 91	0.52	41	*1.0
*163	0.22	42	*2.1
*194	0.78	63	*1.1
*270	0.45	*130	*1.2
*405	0.60	*193	*0.85

*Significantly higher than normal ($p<.05$)

Table 3 compares the electrophoretic patterns of the protein fractions and the protein-bound carbohydrate fractions of pooled control CSF with the CSF of a patient with a primary brain tumor. There is little difference between the electrophoretic protein fractions of the 2 CSF samples. However, there is a striking difference in the distribution of the electrophoretic fractions when stained for carbohydrate with PAS. The analytical system employed was not sufficiently refined to resolve the alpha-2 globulin fraction from the apparent albumin fraction. Therefore, an increase in the alpha-2 globulin fraction would be read as "albumin."

Seven nontumor patients drawn from the "disease control group" whose CSF contained supernormal protein concentration (70 mg. percent) are listed in Table 4 along with their F/H ratios. It is clear from these data that there is no obvious correlation between CSF protein concentration and the F/H ratio even when the CSF contains 6 times the normal amount of protein. Six patients with primary brain tumors are also listed in Table 4 with respect to both their CSF protein concentration and their F/H ratio. It is also clear from these data that, while 4 of these patients had normal CSF protein concentrations, all 6 patients had increased F/H ratios. Again, no correlation could be demonstrated about protein concentration and the F/H ratio.

DISCUSSION

It is assumed that nondialyzable carbohydrates are bound to macromolecules. Two classes of macromolecules (glycoproteins and lipoproteins) are known to contain sialic acid and hexose. Of these two, it is believed only one, glycoprotein, contains fucose. Therefore, an increase in the CSF F/H ratio could be the result either of the release of a unique glycoprotein or of increased amounts of specific glycoproteins normally found in CSF added to the glycoprotein component preexisting in this fluid.

The data of Tables 1 and 2 indicate that

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Tables 1 and 2 indicate that the CSF concentration of nondialyzable hexose is elevated above normal for almost all of the neurologically diseased patients studied. Therefore, the finding is of little diagnostic utility. In spite of this considerable increase in CSF glycoprotein hexose, the F/H ratios in the CSF are essentially normal. This proceeds to a large extent from the fact that in patients without a primary brain tumor, the CSF fucose concentration essentially paralleled that of the hexose. The variations in the CSF sialic acid concentration in patients with neurological diseases were not consistent and did not parallel the hexose concentration. Statistically, only 6% of the 87 "disease control" patients demonstrated a significant elevation in their CSF F/H ratios. In 100% of the 14 patients with a primary brain tumor increased CSF F/H ratios were demonstrated; 40% of the patients with a secondary brain tumor also demonstrated elevated CSF F/H ratios.

The data of these 2 tables suggest strongly that there is a correlation between primary brain tumor and the appearance of a fucose-rich glycoprotein in the CSF.

Analysis of electrophoretic patterns with respect to both protein and carbohydrate of the macromolecular components of the CSF of both pooled disease controls and 1 patient with a primary brain tumor is restricted by our electrophoretic device in that we could not resolve the alpha-1 and alpha-2 globulins from the albumin fraction itself. However, it is clear that the distribution of the protein-staining macromolecular components is not significantly different from the control in the presence of a primary brain tumor. Both the "prealbumin" and the "albumin" electrophoretic fraction from the CSF of the primary brain tumor patient contained supernormal amounts of carbohydrate, however. In spite of the technical limitations, these results, along with the chemical data, do suggest a marked alteration from normal in composition of the glycoprotein in the CSF in a patient with a primary brain tumor.

One possible source of CSF glycoprotein would be the passage of these materials from the circulation across the blood-brain barrier. Serum contains 100 times as much hexose and protein and 20 times as much fucose as does the CSF.[6] Therefore, passage of serum-derived fuco-glycoprotein into the CSF would probably be associated with a paralleled and marked increase in CSF protein. On a milligram basis, 5 times as much hexose and protein per unit weight of fucose would have to move into the CSF. The increased amount of hexose in the CSF of patients with a primary brain tumor was approximately 1 mg. percent. The increased amount of fucose was approximately 2 mg. percent and there was no consistent increment in total protein. This reasoning, coupled with the data of Table 4, which demonstrated no correlation between the CSF protein concentration and the F/H ratio, suggests strongly that either a very specific sequential and active transport of a specific fuco-glycoprotein was involved or the tumor itself was generating a glycoprotein uniquely rich in fucose. Either possibility is suggestive of a hitherto unknown property of primary brain tumors.

The results of this preliminary study are provocative from two points of view: [1] The increased ratio of protein-bound fucose to hexose in the CSF of patients with primary brain tumors might prove to be of some diagnostic value to the clinician. [2] If these results are confirmed in larger groups of patients, they could suggest the existence of some unique property of primary brain tumors related to fuco-glycoprotein metabolism.

SUMMARY AND CONCLUSION

A wide variety of neurological diseases was studied with respect to the concentration of ratios of glycoprotein-derived fucose and hexose in the CSF.

The ratios of fucose to hexose in the CSF glycoproteins in patients with various neurological diseases were not significantly altered from that of the normal controls.

In the CSF of patients with primary brain tumor, there was found an increased ratio of fucose to hexose, a finding of possible diagnostic significance.

This increased (F/H) ratio in patients with primary brain tumors probably does not result from the release of larger amounts of a normally occurring fuco-glycoprotein but rather may result from the appearance in the CSF of a unique abnormal glycoprotein peculiar to the metabolism of primary brain tumor.

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